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The effect of caffeine on the ventilatory response to hypercarbia in preterm infants

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ABSTRACT

Background: We tested the hypotheses that caffeine therapy would increase the ventilatory response to hypercarbia in infants above the effect of maturation and those with a weaker ventilatory response to hypercarbia would be more likely to subsequently develop apnoea which required treatment.

Methods: Infants born less than 34 weeks of gestation underwent a steady state hypercarbic challenge using 0%, 2% and 4% carbon dioxide soon after birth which was repeated at weekly intervals. The results of the initial study were compared between infants who did or did not subsequently develop apnoea requiring treatment with caffeine.

Results: Twenty-six infants born at a median gestation of 32 (range 31-33) weeks were assessed. Caffeine administration was associated with an increase in CO_2 sensitivity, the mean increase was 15.3; (95% CI: 1-30) ml/kg/min/%CO_2. Fourteen infants subsequently developed apnoea treated with caffeine. After controlling for gestational age and birth weight, they had significantly lower carbon dioxide sensitivity at their initial study compared to those who did not require treatment.

Conclusions: Caffeine administration was associated with an increase in the ventilatory response to hypercarbia. An initial weaker ventilatory response to hypercarbia was associated with the subsequent development of apnoea requiring treatment with caffeine.

INTRODUCTION

Apnoea of prematurity is a common problem in prematurely born infants (1) occurring most often in those born very prematurely (2). It is possible that a reduced chemoresponse to hypercarbia may contribute to the development of apnoea (1). Indeed, at approximately one week of age, 18 infants with significant apnoea (a mean of 32 apnoeas lasting longer than 20 seconds per day) had a reduced ventilatory response to carbon dioxide (CO_2) compared to 18 matched controls without apnoea (3).

Caffeine, a methylxanthine, reduces the frequency of apnoeas in preterm infants (4). It increases the ventilatory response to hypercarbia in animals (5) and adults (6, 7). In anaesthetised cats, caffeine administration resulted in an increase in minute ventilation, as well as an increased ventilatory response to inspired CO_2 (5). Administration of theophylline, another methylxanthine, to ten infants with a median gestational age of 30 weeks and apnoea was associated with an increase in minute volume in response to breathing 3% CO_2 and lower end-tidal CO_2 levels (8). The measurements, however, were made prior to and between two and four days after administration of theophylline and no attempt was made to determine whether the effects were due to maturation rather than theophylline administration per se (8). No study has investigated whether caffeine administration influences the ventilatory response to hypercarbia of prematurely born infants. We hypothesised that the ventilatory response to hypercarbia would be increased following administration of caffeine and any effect would be additional to the effect of increasing maturation.

Furthermore, we postulated that those infants who went on to require treatment with caffeine compared to those that did not would, when first studied, have a lower response to a CO_2 challenge. We have undertaken a longitudinal study to test those hypotheses

METHODS

The study was performed at King's College Hospital NHS Foundation Trust between May 2013 and August 2015. Infants were eligible for entry into the study if they were born at less than 34 weeks of gestation, did not require respiratory support and were less than 72 hours of age. Infants were only entered into the study if they had not received caffeine. Infants with major congenital abnormalities were excluded from the study. A hypercapnic challenge was undertaken and repeated at weekly intervals until the infants were discharged from the neonatal unit. Infants who developed clinically significant apnoea (see later) during the course of the study were treated with caffeine. Parent(s) gave informed, written consent for their infant to take part in the study, which was approved by the London Bromley Research Ethics Committee.

The hypercapnic challenge

The test gas was delivered using an open circuit system via a nasal mask and pneumotachograph. The pneumotachograph (Mercury F10L, G M Instruments, Kilwinning, Scotland) which had a dead space 0.8 ml and resistance 0.86 $mmH_2O/L/min$, was connected to a soft latex nasal mask (Neomask, Draeger,

Germany) using a snugly fitting connector. The pneumotachograph was connected to a differential pressure transducer-amplifier system (Gould model 13-4615-70, Cleveland OH) generating an analogue signal proportional to airflow. The mask was placed over the infant's nose. A nasal mask (deadspace 7.5 mls) was used to avoid the stimulation that can result if a facemask is used (9). A seal was achieved by gentle pressure and confirmed by demonstration of no leak (there was no discrepancy between the inspired and expired tidal volumes as displayed in real time on the computer screen. We monitored the flow waveforms to ensure no airway obstruction was occurring. The distal end of the pneumotachograph was connected to a two-way non-rebreathing valve which separated inspired from expired gas, such that the controlled mixture of gases was inspired by the infant and expiration was to the outside air. A constant flow of medical air was delivered to the inspiratory port of the valve via wide bore (20 mm), low resistance tubing to eliminate any dead space. The inspired air could be enriched with a variable concentration of CO_2 from a cylinder. Gas was continuously sampled from the nasal mask through a fine bore catheter at a rate of 180ml/minute using a capnograph (CO_2SMO capnograph; (Respironics UK, Chichester, UK). The CO_2 content of the sampled gas was determined by infrared spectroscopy and an analogue signal proportional to the CO_2 concentration generated. Oxygen saturation was measured using a pulse oximeter (Masimo rainbow SET Pulse Oximetry) attached to the foot of the infant.

Following a five minute period of breathing medical air, the hypercarbic challenge was delivered through the equipment described above. CO_2 was delivered from a cylinder of CO_2 20% balanced with medical air (BOC), via a low-flow meter. Minute volume was measured during exposure to three levels of CO_2 (0% (baseline), 2% and

4%). Those levels were chosen as they have been previously demonstrated to result in changes in respiration without significant behavioural arousal (10-13). Each mixture of CO_2 was titrated using a low flow flowmeter attached to the CO_2 regulator. The capnograph read out was used to determine when a stable inspiratory CO_2 concentration within the delivery tubing had been achieved.

The fraction of inspired oxygen was not monitored or controlled, however, addition of a maximum of 4% CO_2 would result in a potential reduction in the inspired oxygen of less than 1%.

The infant breathed the air/CO_2 mixture for at least five minutes to allow ventilation and $ETCO_2$ to reach a stable state as assessed from the real-time display using the Spectra software (Grove Medical, London, UK). That duration is in keeping with the time used in previous studies (10). Neither transcutaneous CO_2 monitoring nor blood gas analysis was performed. The order of administration of the test gases was randomised between infants. All measurements were made during quiet sleep as determined by Prechtly criteria (14). When arousal occured the measurement was stopped until the infant had returned to quiet sleep and then the measurement began again.

Respiratory flow and gas concentration were acquired and displayed in real time on a PC computer running Spectra software (Grove Medical, London, UK) with 100 Hz analogue to digital sampling (PCI-MIO-16XE-50, National Instruments, Austin TX). Breath by breath data were exported from Spectra software to Microsoft Excel 2011

(Microsoft). Minute volume was calculated from the last minute of exposure at each level of CO_2. CO_2 sensitivity was calculated as the gradient of a line of best fit through a plot of the minute ventilation at each of the three inspired CO_2 levels (0%, 2% and 4% CO_2). CO_2 sensitivity was expressed as ml/kg/min/% CO_2, that is the change in minute volume per 1% change in the inspired CO_2.

Polysomnography

Polysomnography was performed at the initial study to determine whether the infant was having apnoeas. A commercially available Alice 4 sleep study unit (Profile Vio-systems, Bognor Regis, UK), the Alice 5 firmware upgrade was used. Abdominal and thoracic movements were assessed using stretch sensitive piezo-electric respiratory bands. Oral and nasal airflow were measured using the analogue output of the pneumotachograph described above. An electrocardiogram was recorded using single use bipolar electrodes. Two activity meters were attached one to an arm and the other to a leg to record limb movements. Oxygen saturation was continuously monitored using a pulse oximeter (Masimo rainbow SET Pulse Oximetry). The data were incorporated into the Alice sleep system using an auxiliary input.

The Alice sleep system was connected to a PC which was used to display the recording in real time and store data. The infant was monitored by video camera throughout the study with recordings stored on the PC. These recordings were used to assess sleep state and evaluate for artefacts, such as awakenings or handling of the infant. Apnoeas were defined as cessation of respiratory airflow of five seconds in duration (15). This definition has been used in the preterm population (16) and ensures a sensitive assessment of respiratory pauses that may not be detected by other definitions (17).

They were classified as obstructive if there was no airflow despite chest and abdominal wall movements; central if there was no nasal airflow and an absence of chest and abdominal wall movements and mixed if there was a combination of central and obstructive apneas. For each apnoea associated changes in heart rate and oxygen saturations were recorded. The apnoea index (the frequency of apneas per hour) was calculated.

Caffeine administration

The decision to treat with caffeine was made by the lead clinician without knowledge of the results of the polysomnography or hypercarbic challenge. The criteria for treatment with caffeine was apnoea lasting more than twenty seconds or more than ten seconds if associated with oxygen desaturation to less than 90% or bradycardia as demonstrated by routine clinical monitoring (18). A loading dose of 20mg/kg caffeine citrate was administered intravenously, followed by maintenance dose of 10mg/kg every 24 hours given either intravenously or enterally. Caffeine therapy was discontinued at 34 weeks corrected gestational age, or earlier if the infant had been without apnoeas for at least one week.

Data collection

Birth weight, gestational age at birth and maternal age were recorded. Infants were considered to have been exposed to antenatal corticosteroids if at least one dose of corticosteroids was given to their mother 24 hours prior to delivery. A diagnosis of chorioamnionitis was made if the diagnosis was documented in the obstetric records or if the mother had received parenteral antibiotics because of clinical features of chorioamnionitis (fever, fetal tachycardia or offensive liquor). At each study, the

current weight, corrected gestational age and postnatal age and caffeine treatment were documented from the medical records.

Statistical analysis

Results were visually assessed for normal distribution using histograms and normality plots and shown to be normally distributed. Hence differences in outcomes were assessed for statistical significance using Fisher's exact test or the student t-test as appropriate. Comparison of results of the longitudinal measurements of infants receiving or not receiving caffeine were performed using a paired t-test.

Linear mixed models with fixed effects were developed to account for repeated measures, with carbon dioxide sensitivity as the dependent variable. Corrected gestational age was entered as a covariate, when determining the effect of corrected gestational age on CO_2 sensitivity, with caffeine as a factor to determine the additional effect of caffeine on CO_2 sensitivity.

Differences in results of the initial challenge between those who that went on to require treatment with caffeine and those that did not were assessed using regression analysis. Adjustment was made for birthweight and gestational age by fitting those variables as covariates. Adjusted means are marginal estimates for the dependent variable derived from the regression model at the mean value of the covariates: birth weight and gestational age.

Analyses were conducted using SPSS 22 (IBM).

SAMPLE SIZE

A sample size of two groups of 15 would allow detection of a two-sided difference between the caffeine and non treated infants of a difference equivalent to one standard deviation to be detected by 90% power. That difference had been detected in the ventilatory response to added dead space (primarily a hypercarbic challenge) between newborns of smoking and non-smoking mothers (19).

RESULTS

Twenty-six infants born at a median gestation of 32 (range 31-33) weeks and a median birth weight 1590 (range 840 – 2200) gms were recruited into the study. They were first studied at a median 22 (range 6-69) hours after birth. Ninety-two studies, each comprising exposure to the three levels of CO_2, were performed with a median of 4 (range 1-6) studies per infant. Fourteen infants developed significant apnoea and were treated with caffeine (table 1). The infants who went on to develop significant apnoea were significantly lower birth weight ($p=0.03$) (Table 1). At the initial study, there was no difference in the apnoea index between those infants that went on to develop significant apnoea and those that did not (Table 2). CO_2 sensitivity was significantly higher following caffeine administration (n=14; mean difference 41ml/kg/min/% CO_2 95% CI 26-57; $p<0.001$) the measurements were made one week apart (Figure 1). Baseline minute volume while breathing air did not differ significantly between the two measurements. (Figure 2)

CO_2 sensitivity was significantly lower after discontinuing caffeine therapy (n=11; mean difference 15 ml/kg/min/%CO_2 95% CI 1-29; p=0.036) (Figure 3). The measurements were made one week apart.

A linear mixed model of results from infants who did not receive caffeine (n=12) demonstrated that increased corrected gestational age resulted in an increase in carbon dioxide sensitivity. A one week increase in corrected gestational age correlated with an increase in carbon dioxide sensitivity of 9.5ml/kg/min/%CO_2 (95% CI: 4.3-14.6; p<0.001). When all the infants were included (n=26) in the analysis and caffeine introduced as a factor, corrected gestational age and caffeine both significantly contributed to a linear mixed model for CO_2 sensitivity. The estimated effect of corrected gestational age was an increase of 10.0ml/kg/min/%CO_2 (95% CI: 5.4-14.5; p<0.001) per week. The estimated marginal mean effect of caffeine therapy on CO_2 sensitivity at 33 weeks of gestation was 15.3 ml/kg/min/%CO_2; (95% CI: 1-30; p=0.042).

Infants who subsequently developed significant apnoea had significantly lower birth weights (p=0.03) and a trend towards lower gestational ages (p=0.06) and lower carbon dioxide sensitivities (p=0.065) (Tables 1 and 2). After controlling for the effect of corrected gestational age and birth weight, carbon dioxide sensitivity at initial study was significantly lower in those infants that went on to develop apnoea requiring treatment with caffeine compared to those that did not (Table 2).

DISCUSSION

We have demonstrated that caffeine administration was associated with an increase in the ventilatory response to hypercarbia in prematurely born infants and that the effect remained significant after controlling for increasing maturity. When caffeine was discontinued, CO_2 sensitivity significantly decreased, further suggesting that the effect was due to caffeine administration per se. Methylxanthines are phosphodiesterase inhibitors and, at therapeutic concentrations, are non-specific adenosine receptor antagonists. Adenosine receptors are expressed throughout the brain stem respiratory centres. There are several subtypes, blockade of which have varying effects on respiratory control (20-22). Adenosine A2A receptors play a role in the development of late hypoxic ventilatory depression, while A1 receptors contribute to cardiorespiratory control during normoxia (23). The role of these receptors changes with development.

The response to the initial hypercarbic challenge was significantly weaker in those who went on to develop significant apnoea than in those that did not. Those findings are consistent with the reports that a weaker response to hypercarbia was found in infants with significant apnoea (3). Furthermore, Durand et al. compared eight preterm infants with at least three episodes a day of apnoea lasting more than 20 seconds to nine preterm infants without apnoea and reported reduced baseline ventilation and CO_2 sensitivity in those with apnoea (24). Apnoea resulting in chronic intermittent hypoxia, however, may modulate the subsequent ventilator response to hypercapnoea (25). Exposure of neonatal piglets to intermittent hypercapnia and hypoxia reduced the subsequent ventilatory response to hypercapnia (26). Two

studies, however, in rat pups using several different regimes of hypercapnic or normocapnic hypoxia failed to demonstrate a significant effect on the subsequent ventilatory response to hypercapnia (27, 28). Millstrom exposed newborn rat pups to regimes of intermittent hypercapnic and hypoxia (6% CO_2/10% O_2), or intermittent hypercapnic and hypoxia alternating with hyperoxia (30% O_2), five times an hour over the first two weeks after birth. Neither regime had any subsequent effect on the ventilatory response to hypercarbia (27). Peng used a shorter duration of intermittent hypoxia (15 seconds of 5% O_2, nine times an hour for eight hours a day during the first ten days after birth in newborn rats and again showed no significant difference in the their hypercapnic response compared to non-exposed controls (28). Importantly, our study has suggested that a reduced carbon dioxide sensitivity in infants may precede the development of significant apnoea and is, therefore, more likely to be a contributive factor rather than a result of recurrent apnoea induced episodes of hypercarbia and hypoxia. Whether antenatal episodes of hypercarbia may contribute is unclear. Those infants that went on to develop significant apnoea were significantly more likely to be SGA. Poor growth may reflect impairment of placental blood flow. Therefore, exposure to periods of intermittent hypoxia antenatally could potentially contribute to an impairment of CO_2 sensitivity.

This study has strengths and some limitations. A strength of our study is that we undertook longitudinal measurements, including infants who did not require caffeine treatment. Hence, we have been able to control for the maturational effect on the ventilatory response to hypercapnia. Our study required recruitment of infants born sufficiently prematurely that they were likely to develop apnoea, but did not require respiratory support. This, then, was a highly selective group. Unfortunately we did not

recruit the target number from the sample size calculation. Nevertheless, we were able to demonstrate significant differences between groups and given the numbers recruited we were able to detect differences with 79% power. We did not measure caffeine levels. Absorption and metabolism of caffeine is variable in premature infants, and therefore may have introduced heterogeneity into the response to caffeine therapy. Nonetheless, the effect of caffeine on CO_2 sensitivity remained significant.

In conclusion, caffeine administration was associated with an increase in the ventilatory response to hypercarbia. In addition, carbon dioxide sensitivity assessed soon after birth was significantly lower in those infants who subsequently required treatment with caffeine for apnoea.

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FIGURE LEGENDS

Figure 1: Box plot showing carbon dioxide sensitivity before and after administration of caffeine. Box plot shows median and interquartile range. Whiskers extend to 1.5 times the interquartile range. Data points beyond the interquartile range are marked as outliers.

Figure 2: Boxplot showing minute volume while breathing air before caffeine therapy was initiated and one week later. Box plot shows median and interquartile range. Whiskers extend to 1.5 times the interquartile range. Data points beyond the interquartile range are marked as outliers.

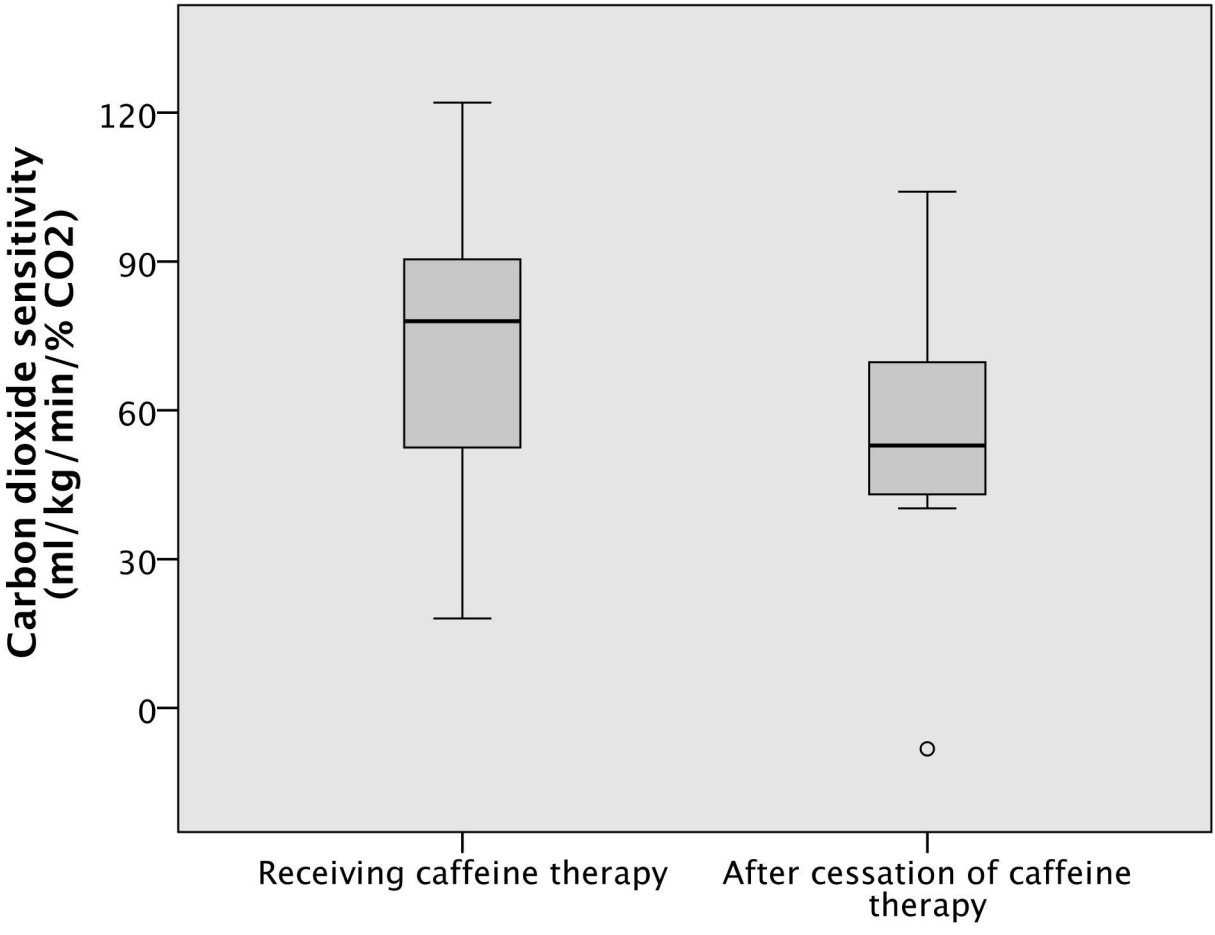
Figure 3: Boxplot showing carbon dioxide sensitivity while receiving caffeine therapy and carbon dioxide sensitivity at the following measurement one week later, following cessation of caffeine therapy. Box plot shows median and interquartile range. Whiskers extend to 1.5 times the interquartile range. Data points beyond the interquartile range are marked as outliers.

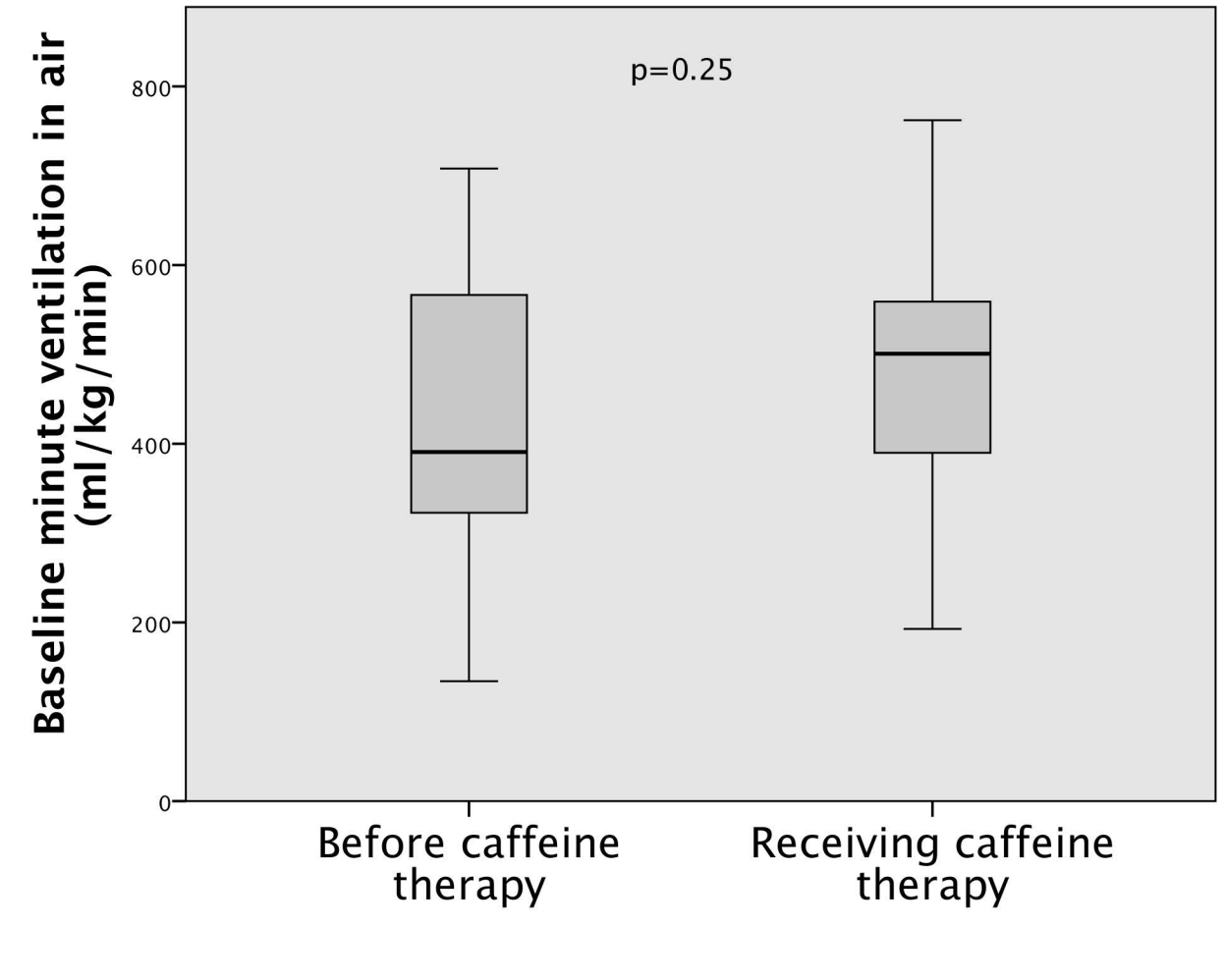
Table 1: Demographics of study population according to the development of significant apnoea.
Data are presented as mean (standard deviation) if normally distributed; median (range); or n

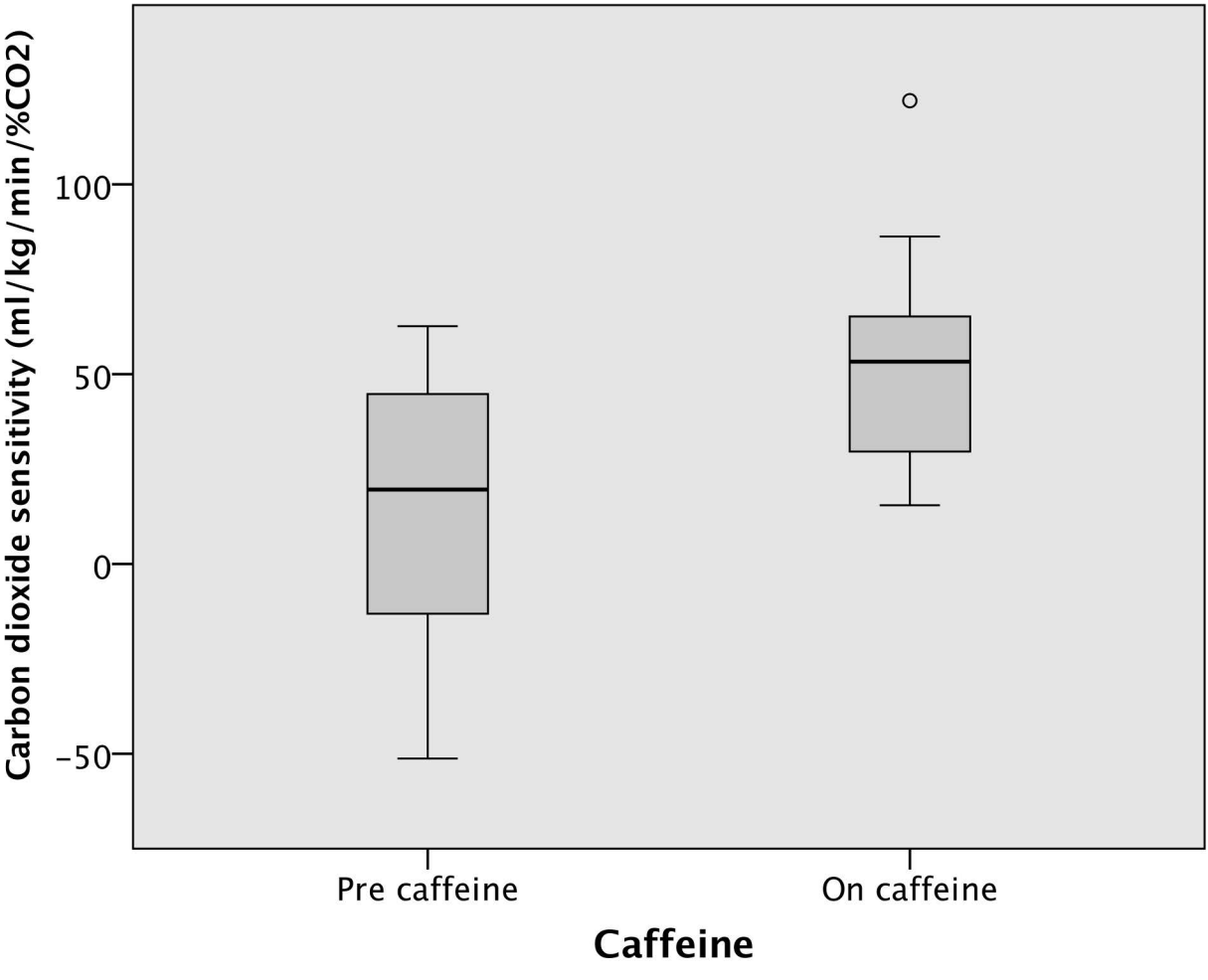
	No significant apnoea	Significant apnoea	p-value
n	12	14	
Birthweight (g)	1730 (340)	1450 (290)	0.03
Gestational age (weeks)	32.7 (0.5)	32.2(0.7)	0.06
Maternal age (years)	32 (7)	30 (6)	0.45
Antenatal steroids	10	13	0.58
Chorioamnionitis	1	3	0.60
Caesarean section	10	10	0.65
Sex (F)	6	5	0.69
Birth weight <10th centile	3	6	0.43
Singleton	10	9	0.39
Apgar at 5 minutes	10 (9-10)	9 (8-10)	0.37
Supplementary oxygen >12 hours	7	7	0.71
Positive pressure support >12 hours	3	5	0.68

Table 2: Results of the initial study, grouped according to significant apnoea status
Data are presented as mean (SD)
* Denotes marginal means adjusted for birth weight and corrected gestational age

	No significant apnoea	Significant apnoea	p-value
n	12	14	
Baseline minute volume (ml/kg/min)			
mean (SD) unadjusted	431 (91)	426 (165)	0.92
mean (95% CI) adjusted*	446 (356-537)	413 (330-496)	0.61
Baseline end-tidal CO_2 (%)			
mean (SD) unadjusted	4.1 (0.7)	4.4 (0.8)	0.22
mean (95% CI) adjusted*	4.1 (3.6-4.6)	4.4 (4.0-4.9)	0.36
Apnoea index (events/hr)			
mean (SD) unadjusted	3.8 (3.6)	3.1 (2.4)	0.61
mean (95% CI) adjusted*	4.1 (1.7-6.5)	2.9 (0.4-5.4)	0.47
CO_2 sensitivity (ml/kg/min/%CO_2)			
mean (SD) unadjusted	34 (15)	13 (36)	0.07
mean (95% CI) adjusted*	38 (19-57)	9 (-8-27)	0.04


Carbon dioxide sensitivity (ml/kg/min/% CO2)
120
90
60
30
0
Receiving caffeine therapy
After cessation of caffeine therapy


Baseline minute ventilation in air (ml/kg/min)
p=0.25
800
600
400
200
0
Before caffeine therapy
Receiving caffeine therapy


Carbon dioxide sensitivity (ml/kg/min/%CO2)
100
50
0
-50
Pre caffeine
On caffeine
Caffeine